FINAL REPORT FOR AIR FORCE OFFICE OF SCIENTIFIC RESEARCH GRANT #F49620-97-1-0217-P00001 FOR THE PERIOD OF MAY 15, 1997 TO MAY 14, 2000.

THE ROLE OF SUBSTANCE P IN A MODEL OF CHRONIC JP-8 JET FUEL EXPOSURE

Please make extra copy for me

WJK

Mark L. Witten, Ph.D.
Principal Investigator
Department of Pediatrics
Arizona Health Sciences Center
1501 N. Campbell Avenue
Tucson, Arizona 85724

Report Submitted on June 9, 2000.

REPORT DOCUMENTATION PAGE

AFRL-SR-BL-TR-00-

0439

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
		Final - 15 May 1997 - 14 May 2000

4. TITLE AND SUBTITLE
The Role of Substance P in a Model of Chronic JP-8 Jet Fuel

5. FUNDING NUMBERS
F49620-97-1-0217

6. AUTHOR(S)
Dr. Mark L. Witten
Department of Pediatrics

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Arizona
Tucson, AZ 85724-0001

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING/MONITORING AGENCY NAME(S) AND ADDRESS(ES)
AFOSR/NL
801 North Randolph Street, Rm 732
Arlington, VA 22203-1977

10. SPONSORING/MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES

12a. DISTRIBUTION AVAILABILITY STATEMENT
APPROVED FOR PUBLIC RELEASE: DISTRIBUTION UNLIMITED

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

We have established a good dose-response relationship for JP-8 jet fuel exposure in our mouse model and further elucidated the role(s) of substance P in the lung injury cascade initiated by JP-8 jet fuel exposure. Additionally, we have established good working relationships with Drs. Harris, Witzmann and Siegel and these relationships are becoming productive in producing addtional JP-8 jet fuel-related research. We will establish a mouse viral model in the next three years of support from AFOSR as well as continue our studiesof SP in this model. We will ascertain whether a pre-existing respiratory infection followed by JP-8 jet fuel exposure is synergetic to cause significant lung injury. Additionally, we will examine the lung proteomics of a minimum JP-8 jet fuel dose, 50mg/m, that we know to cause pathological lung injury in collaboration with Dr. Witzmann.

14. SUBJECT TERMS
JP-8 Fuel

15. NUMBER OF PAGES
10

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclas	Unclas	Unclas	

BACKGROUND

The past three years of our studies of the effect(s) of JP-8 jet fuel exposure on the pulmonary system has resulted in four published manuscripts, three submitted manuscripts, and numerous abstracts from the Lung Injury Laboratory. Additionally, research with substance P (SP) and its attenuation of JP-8 jet fuel-induced lung injury and immunostimulatory properties has lead to two United States patents, one Australian patent, and a submitted European Union patent application. Furthermore, we have developed a lung biosensor based on a stable component of surfactant that can detect JP-8 jet fuel vapors. The University of Arizona has filed a provisional patent application with the United States Patent & Trademark Office for our lung biosensor invention.

Finally, we have supported other Air Force Office of Scientific Research sponsored investigators by performing specific JP-8 jet fuel exposure trials. This research has resulted in 10 publications in areas such as immunology and proteomics.

RESULTS

(1) Robledo RF, Witten ML: Acute pulmonary response to inhaled JP-8 jet fuel aerosol in mice. INHALATION TOXICOLOGY, 1998, 10:531-553.

The pulmonary response to JP-8 jet fuel inhalation was investigated by characterizing biomarkers of lung injury, respiratory permeability, pulmonary function, and lung morphology. The JP-8 jet fuel concentrations ranged from 0 to 113 mg/m^3 of aerosolized JP-8 jet fuel for one hour. After the single JP-8 jet fuel exposure, the mice were killed 24-30 hours later. The single JP-8 inhalation exposure did not have an effect on pulmonary function even though histopathology data showed evidence of terminal bronchiole lesions. These results indicate that a single JP-8 exposure within NAVOSH permissible personnel exposure levels can cause changes in lung permeability and BALF markers of lung injury.

(2) Robledo RF, Witten ML: NK_1-receptor activation prevents hydrocarbon-induced lung injury in mice. AMERICAN JOURNAL OF PHYSIOLOGY (Lung Cell Mol Physiol) 276:L229-L238, 1999.

We investigated the effect(s) of NK_1-receptor activation after inhalation exposure to JP-8 jet fuel for one hour/day for seven days. Lung injury was assessed by the analysis of pulmonary function, bronchoalveolar lavage fluid (BALF), and lung morphology. JP-8 jet fuel exposure, 50 mg/m^3, was characterized by increased lung permeability to ^{99m}Tc-labeled diethylenetriaminepentaacetic acid. Mice administered the SP agonist, Sar^9, Met $(O_2)^{11}$-SP, after each JP-8 exposure had the appearance of normal pulmonary values and tissue morphology. In contrast, endogenous NK_1-receptor antagonism by CP-96345 administration exacerbated JP-8 jet fuel-induced lung permeability, alveolar macrophage toxicity, and bronchiolar epithelial injury. These data indicate that NK_1-receptor activation may have a protective role in preventing the development of hydrocarbon-induced lung injury, possibly through the modulation of bronchiolar epithelial function.

(3) Robledo RF, Barber DS, Witten ML: Modulation of bronchial epithelial cell barrier function by in vitro jet propulsion fuel 8 exposure. TOXICOLOGICAL SCIENCES, 51:119-125, 1999.

We studied whether the loss of epithelial barrier integrity in bronchial and bronchiolar airways may be an initiating factor in the observed onset of JP-8 jet fuel-induced lung injury. We utilized BEAS-2B human bronchial epithelial cells in this study and measured the extent of JP-8 jet fuel-induced changes in bronchial epithelial barrier function by mannitol flux. Incubation of confluent cell cultures with non-cytotoxic concentrations of JP-8 jet fuel or n-tetradecane (C_{14}), a primary constituent of JP-8, for a one hour exposure period resulted in dose-dependent increases of paracellular flux. Following exposures of 0.17, 0.33, 0.50, or 0.67 mg/ml, mannitol flux increased above vehicle controls by 10, 14, 29, and 52%, respectively, during a 2 hour incubation period immediately after each JP-8 jet fuel exposure. C_{14} caused greater mannitol flux increases of 37, 42, 63, and 78%, respectively, following identical exposure conditions. The effect of transepithelial mannitol flux reached a maximum at 12 hours and spontaneously reversed to control values over a 48-hour recovery period, for both JP-8 and C_{14} exposures. These data demonstrate that non-cytotoxic exposures to JP-8 jet fuel or C_{14} exert a

noxious effect on bronchial epithelial barrier function that may preclude pathological lung injury.

(4) Robledo RF, Young RS, Lantz RC, Witten ML: Short-term pulmonary response to inhaled JP-8 jet fuel aerosol in mice. TOXICOLOGIC PATHOLOGY, (in press).

B6.A.D. (Ahr[d]/Nat[s]) mice were utilized to investigate the short-term pulmonary response to JP-8 jet fuel aerosol inhalation. Mice were nose-only exposed to atmospheres of 0 to 118 mg/m^3 for one hour/day over a period of seven days, to further test the hypothesis that JP-8 concentrations below the permissible exposure level (PEL) of 350 mg/m^3 will induce pathological lung injury. The electron microscopic analyses demonstrated changes in lung alveolar epithelium with as little as 50 mg/m^3 JP-8 jet fuel exposure.

(5) Young RS, Witten ML: Alteration of pulmonary function and lung permeability in aged mice after exposure to JP-8 jet fuel. TOXICOLOGICAL SCIENCES, (submitted).

We conducted studies to determine the age-related effect(s) of JP-8 + 100 blend jet fuel inhalation on the pulmonary microenvironment in aged animals. We utilized two groups of mice in this study, aged (12-14 months) and young (3-4 months) mice to 1000 mg/m^3 JP-8 + 100 blend jet fuel for seven days for one hour/day. The animals were sacrificed 24-30 hours after the final JP-8 jet fuel exposure and pulmonary functions, lung permeability, and bronchoalveolar lavage fluid (BALF) were analyzed. Aged mice exposed to 1000 mg/m^3 JP-8 + 100 blend jet fuel demonstrated significant alterations in pulmonary functions and lung permeability (Table 3), and BALF levels of inflammatory mediators and lipid peroxidation products (Figures 1-4) compared to aged controls and young mice exposed to JP-8 + 100 blend jet fuel.

(6) Young RS, Witten ML: JP-8 jet fuel-induced alterations in primed pulmonary alveolar macrophages. TOXICOLOGICAL SCIENCES. (submitted).

This study examined, in vitro, the effects of JP-8 jet fuel on alveolar type II (AEII) and pulmonary alveolar macrophage (PAM) function. We studied AEII cells alone and in co-culture with primary PAM as well as the effects of JP-8 jet fuel on PAM before and after priming with lipopolysaccharide (LPS). Our results demonstrate that JP-8 jet fuel induces significant alterations to AEII cells and that the lungs' defensive

response to JP-8 jet fuel exposure may be mediated by alterations in PAM activity. Studies from LPS-primed PAM demonstrate a decreased ability to induce an immune response as measured by an attenuated ability to secrete tumor necrosis factor-alpha and interleukin-10. This decreased function in JP-8 jet fuel-exposed PAM may promote lung injury by other aeroallergens and increase the susceptibility to pulmonary infections from opportunistic pathogens.

(7) Baldwin CM, Houston FP, Podgornik MN, Young RS, Barnes CA, Witten ML: Effects of aeroso-vapor JP-8 jet fuel on the functional observational battery, and learning and memory in the rat. ARCHIVES OF ENVIRONMENTAL HEALTH (in press).

We studied the effect(s) of JP-8 jet fuel exposure on visual discrimination, spatial learning and memory, and the functional observational battery. The male Fischer Brown Norway hybrid rats were exposed to 28 days of aerosol/vapor delivered JP-8 or JP-8 followed by 15 minutes of aerosolized substance P (SP) analogue, or sham-confined fresh room air. Behavioral testing was accomplished with the EPA-endorsed functional observational battery (FOB). Visual and spatial learning and memory was tested using the Morris swim task. The spatial test included examination of memory for the original target location after 15 days of JP-8 jet fuel exposure, as well as a three-day new target location learning paradigm implemented the day after the last day of exposure. JP-8 only exposed animals had a significant amount of weight loss by the second week of exposure compared to JP-8 + SP and control rats, a finding consistent with prior studies of JP-8 jet fuel. Animals exposed to JP-8 with or without SP exhibited significantly greater rearing and less grooming behavior over time than did controls during FOB open field testing. Exposed rats also swam significantly faster compared to control animals during the new target location training and testing, supporting the increased activity noted during FOB testing. There were no significant differences between exposed and control group performance during acquisition, retention or learning of the new platform location in either the visual discrimination or spatial version of the Morris swim task. These data suggest that although visual discrimination and spatial learning and memory are not disrupted by JP-8 jet fuel exposure, arousal indices and activity measures were distinctly different in these animals.

PUBLICATIONS FOR GRANT PERIOD-

(1) Harris DT, Sakiestewa D, Robledo RF, Witten M: Immunotoxicological effects of JP-8 jet fuel exposure. TOXICOLOGY & INDUSTRIAL HEALTH, 1997, 13:43-55.

(2) Harris DT, Sakiestewa D, Robledo RF, Witten M: Protection from JP-8 jet fuel induced immunotoxicity by administration of aerosolized substance P. TOXICOLOGY & INDUSTRIAL HEALTH, 1997, 13:571-588.

(3) Robledo RF, Witten ML: [Sar^9, Met $(O_2)^{11}$]-Substance P may protect against JP-8 jet fuel-induced lung injury via increased JP-8 lung clearance. PROCEEDINGS OF THE 1997 TACHYKININS IN HEALTH & DISEASE INTERNATIONAL CONFERENCE, Cairns, Australia, 1997, pp. 40.

(4) Witten ML, Harris DT, Robledo RF, Srinivasan D: Aerosolized [Sar^9, Met $(O_2)^{11}$]-Substance P causes immunostimulation in three different animal models. PROCEEDINGS OF THE 1997 TACHYKININS IN HEALTH & DISEASE INTERNATIONAL CONFERENCE, Cairns, Australia, 1997, pp. 19.

(5) Harris DT, Sakiestewa D, Robledo RF, Witten M: Short-term exposure to JP-8 jet fuel results in longterm immunotoxicity. TOXICOLOGY & INDUSTRIAL HEALTH, 1997, 13:559-570.

(6) Robledo RF, Witten ML: Acute pulmonary response to inhaled JP-8 jet fuel aerosol in mice. INHALATION TOXICOLOGY, 1998, 10:531-553.

(7) Baldwin CM, Podgornik MN, Young RS, Rao G, Houston F, Barnes CA, Witten ML: Biobehavioral and memory alterations related to moderate and high dose JP-8 jet fuel exposure. PROCEEDINGS OF THE 1998 INTERNATIONAL CONFERENCE ON THE ENVIRONMENTAL HEALTH & SAFETY OF JET FUEL, San Antonio, Texas, pp. 55-56.

(8) Witten ML, Harris DT, Robledo RF, Pfaff JK, Hays AM, Young RS: JP-8 jet fuel exposure causes lung injury. PROCEEDINGS OF THE 1998 INTERNATIONAL CONFERENCE ON THE ENVIRONMENTAL HEALTH & SAFETY OF JET FUEL, San Antonio, Texas, pp. 46.

(9) Kornguth S, Wright L, Witten M, Siegel F: Effect of JP-8 fuel aerosol on glutathione-s transferase levels in retina and cerebellum of swiss webster mice. PROCEEDINGS OF THE 1998 INTERNATIONAL CONFERENCE ON THE ENVIRONMENTAL HEALTH & SAFETY OF JET FUEL, San Antonio, Texas, pp. 47.

(10) Harris D, Sakiestewa D, Robledo R, Witten M: Immunotoxicological effects of exposure to JP-8 jet fuel. PROCEEDINGS OF THE 1998 INTERNATIONAL CONFERENCE ON THE ENVIRONMENTAL HEALTH & SAFETY OF JET FUEL, San Antonio, Texas, pp. 48-49.

(11) Witzmann F, Fultz C, Young R, Witten M, Wright L, Kornguth S, Siegel F: Tissue/blood biomarkers: two-dimensional protein mapping. PROCEEDINGS OF THE 1998 INTERNATIONAL CONFERENCE ON THE ENVIRONMENTAL HEALTH & SAFETY OF JET FUEL, San Antonio, Texas, pp. 50-51.

(12) Robledo RF, Witten ML: NK1 receptor activation prevents hydrocarbon-induced lung injury in mice. PROCEEDINGS OF THE NATO BIOACTIVE PEPTIDES CONFERENCE, Palermo, Sicily (Italy), 1998.

(13) Witten ML, Sridhar KR: The utilization of Mars exploration technology in biomedical research. PROCEEDINGS OF THE INTERNATIONAL CONFERENCE ON INTEGRATED NANO/ MICROTECHNOLOGY FOR SPACE APPLICATIONS, Houston, Texas, 1998, pp. 105.

(14) Robledo RF, Witten ML: NK1 receptor activation prevents hydrocarbon-induced lung injury in mice. AMERICAN JOURNAL OF PHYSIOLOGY: LUNG CELLULAR AND MOLECULAR PHYSIOLOGY, 1999, 276:L229-L238.

(15) Robledo RF, Barber DS, Witten ML: Modulation of bronchial epithelial cell barrier function by *in vitro* jet-propulsion fuel 8 exposure. TOXICOLOGICAL SCIENCES, 1999, 51:119-125.

(16) Robledo RF, Witten ML: Short-term pulmonary response to inhaled JP-8 jet fuel aerosol in mice. TOXICOLOGIC PATHOLOGY (in press).

(17) Witzmann FA, Bauer MD, Fieno AM, Fultz CD, Grant RA, Keough TW, Kornguth SE, Lacey MP, Siegel FL, Sun Y, Wright LS, Young RS, Witten ML: Proteomic analysis of simulated occupational jet fuel exposure in the lung. ELECTROPHORESIS, 1999, 20: 3659-3669.

(18) Kornguth S, McGuire S, Wright L, Bostad E, Nelson S, Daggett D, Witten M, Siegel F: Increased immunoreactivity of glutathione -s-transferase in retina of Swiss-Webster mice following inhalation of JP-8+100 aerosols. ARCHIVES OF TOXICOLOGY (in press).

(19) Witzmann FA, Bauer MD, Fieno AM, Fultz CD, Grant RA, Keough TW, Kornguth SE, Lacey MP, Siegel FL, Sun Y, Wright LS, Young RS, Witten ML: Proteomic analysis of simulated occupational jet fuel exposure in the kidney. ELECTROPHORESIS (in press).

(20) Harris DT, Sakiestewa D, Titone D, Robledo RF, Young RS, Witten M: Jet fuel-induced immunotoxicity. TOXICOLOGY & INDUSTRIAL HEALTH (in press).

(21) Harris DT, Sakiestewa D, Robledo RF, Young RS, Witten M: Effects of short term JP-8 jet fuel exposure on cell-mediated immunity. TOXICOLOGICAL SCIENCES (submitted).

(22) Harris DT, Sakiestewa D, Titone D, Robledeo RF, Young RS, Witten M: Analysis of the protective effects of substance P on JP-8 jet fuel-induced immunotoxicity. TOXICOLOGICAL SCIENCES (submitted).

(23) Baldwin CM, Houston FP, Podgornik MN, Young RS, Barnes CA, Witten ML: Effects of aerosol-vapor JP-8 jet fuel on the functional observational battery, and learning and memory in the rat. ARCHIVES OF ENVIRONMENTAL HEALTH (in press).

INVENTIONS FOR GRANT PERIOD-

(1) U.S. Patent Number 5945508, "Substance P for Treatment of Immunostimulation". This patent covers the anti-cancer properties of substance P.

(2) U.S. Patent Number 5998376, "Substance P for Treatment of Immunostimulation". This patent covers the immunostimulatory properties of substance P.

(3) Australian Patent Granted, "Substance P for Treatment of Immunostimulation".

(4) European Union and Canadian patent applications pending.

(5) The University of Arizona filed a provisional patent application entitled, "A Reusable Lung Biosensor for Hydrocarbon Measurements" with the U.S. Patent & Trademark Office on December 9, 1999. I have been contacted by three U.S. venture capital companies and one German company with interest in commercializing this invention.

SUMMARY

We have established a good dose-response relationship for JP-8 jet fuel exposure in our mouse model and further elucidated the role(s) of substance P in the lung injury cascade initiated by JP-8 jet fuel exposure. Additionally, we have established good working relationships with Drs. Harris, Witzmann, and Siegel and these relationships are becoming productive in producing additional JP-8 jet fuel-related research.

We will establish a mouse viral model in the next three years of support from the Air Force Office of Scientific Research as well as continue our studies of SP in this model. We will ascertain whether a pre-existing respiratory infection followed by JP-8 jet fuel exposure is synergistic to cause significant lung injury. Additionally, we will examine the lung proteomics of a minimum JP-8 jet fuel dose, 50 mg/m^3, that we know to cause pathological lung injury in collaboration with Dr. Witzmann.

OBJECTIVE

The Lung Injury Laboratory at the University of Arizona serves as the JP-8 jet fuel animal exposure center for five separate Air Force Office of Scientific Research-funded research projects. Consequently, the workload for the animal exposures has increased exponentially due to differences in animal species (mice and rats), different JP-8 exposure levels, and different JP-8 jet fuel exposure regimens. In order to facilitate a more efficient JP-8 jet fuel exposure operation for the five different research projects, we believe it is necessary to purchase another IN-TOX inhalation exposure system.

SUMMARY

The Lung Injury Laboratory purchased a state-of-the-are inhalation exposure chamber from IN-TOX, Inc. in Albuquerque, New Mexico. This inhalation chamber is working well and allows us to continue to serve as the JP-8 jet fuel exposure center for five separate Air Force Office of Scientific Research-funded research projects concerning acute and chronic exposure to JP-8 jet fuel.